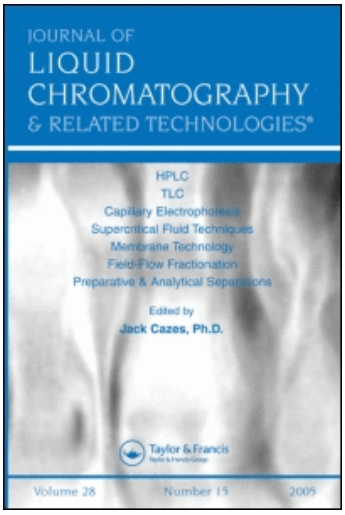

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Górecki, Tadeusz , Panić, Ognjen and Oldridge, Nathan(2006) 'Recent Advances in Comprehensive Two-Dimensional Gas Chromatography (GC×GC)', Journal of Liquid Chromatography & Related Technologies, 29: 7, 1077 — 1104
To link to this Article: DOI: 10.1080/10826070600574762
URL: http://dx.doi.org/10.1080/10826070600574762

Journal of Liquid Chromatography & Related Technologies®, 29: 1077–1104, 2006

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070600574762

Recent Advances in Comprehensive Two-Dimensional Gas Chromatography (GC × GC)

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Abstract: Fourteen years after its introduction, comprehensive two-dimensional gas chromatography (GC × GC) is one of the most powerful analytical tools available for the analysis of very complex samples. The technique is based on the application of two GC columns coated with different stationary phases, connected in series through a special interface (modulator). The interface not only physically connects the two columns, but also ensures the preservation of the separation achieved in the first separation dimension. Compounds coeluting from the first column undergo additional separation in the second column. This leads to vastly increased separation power of the technique compared to conventional 1D GC. This paper reviews the development of the technique, focusing on the most recent advances in the GC × GC field. It describes the principles of the technique, the basics of data processing, instrumentation development, and selected applications.

Keywords: Recent advances, Two-dimensional gas chromatography (GC × GC)

INTRODUCTION

Since its invention and implementation, chromatography has evolved into an invaluable tool for chemical separation and identification of the components of complex samples. It has found innumerable applications in many different areas. Techniques like gas chromatography (GC), high performance liquid chromatography (HPLC), or thin-layer chromatography (TLC) are routinely used, both to answer everyday questions and to solve complex scientific problems.

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In spite of the great separation power of conventional, one-dimensional modern chromatographic techniques, some samples are simply too complex to be effectively analyzed by these methods. Examples of analyses of very complex mixtures can be found in many different areas, including the petroleum industry, where proper characterization of different petroleum fractions and/or products is of critical importance; the forensic sector, striving to improve pattern recognition within complex matrices; as well as the food and fragrance industry, environmental and health sectors, where more sensitive and selective trace analysis of target analytes in complex matrices is required, together with the ability to fully characterize unknown samples. In all of the above examples, it is usually impossible to achieve complete separation of all sample components by relying on a single separation mechanism. This has been the thrust behind the push towards the development of multi-dimensional chromatographic techniques. In his seminal paper published in 1984, Giddings introduced fundamental definitions and described the criteria for multidimensional separations.[1] By this time, multidimensional separations were used in many areas of separation science.[2] In the case of gas chromatography, multidimensional separations initially had the form of heart-cutting techniques, in which a fraction (or several consecutive fractions) of the effluent from the first column were injected into a secondary column coated with a different stationary phase.[3] Such techniques allow for additional separation of the components eluting in selected regions of the chromatograms, and as such are particularly useful in target analysis, provided that the number of target analytes is modest.

While heart-cutting techniques are still successfully used for many applications, they are unable to solve many of the problems mentioned above on their own. Coupling of gas chromatography to mass spectrometry (MS) is another way to add a dimension to analyte separation by providing mass spectrum for every chromatographic peak eluting from the GC column.[4] However, even with the spectral deconvolution ability of powerful modern MS detectors, such as the time-of-flight mass spectrometer (TOF-MS), separation and identification of the components of very complex mixtures often remains unsatisfactory. Therefore, the key to improving the separation power seems to lie primarily in the multidimensionality of the chromatographic separation itself. In particular, it is important that the multidimensional separation is comprehensive. Giddings defined a comprehensive multidimensional separation as one in which the entire sample is subject to all dimensions of separation, and any subsequent separation dimension preserves the separation obtained in the previous separation dimension. In gas chromatography, this implies subjecting the sample to a separation system containing two different columns connected in series, in such a way that the separation achieved in the first column is not lost in the second column. The first practical implementation of this principle was demonstrated in 1991 by Phillips,[5] who integrated a special interface (modulator) between the two columns to accomplish this goal. In this way, the technique of

comprehensive multidimensional gas chromatography, or GC × GC, was born. The following years witnessed a rapid development of this technique and its implementation in many branches of industry and various research sectors, owing to dramatically increased resolution, sensitivity, peak capacity, and selectivity of the separations performed by this technique. This paper focuses mostly on recent developments in the area of GC × GC. Readers seeking a more detailed picture of the evolution of GC × GC are advised to refer to literature reviews published earlier (e.g., refs.[4,6]).

GC × GC FUNDAMENTALS

Principles of the Technique

Multidimensional separations are the easiest to implement in planar chromatography. For example, in thin-layer chromatography (TLC), the sample is spotted on one corner ofz the TLC plate and developed with a mobile phase of choice. The mobile phase transports the analytes upward, resulting in their separation based on the interactions with the mobile and the stationary phase. The chromatogram obtained at this stage is a series of spots arranged along a vertical line. To accomplish two-dimensional separation, the plate is dried, rotated by 90° to position the analyte spots at the bottom, and developed with a different mobile phase. In this way, the spots obtained in the first separation can be further separated in the second dimension. In the resulting chromatogram, the spots are distributed on the entire plane rather than along a line. This dramatically increases the resolution, separation power, and peak capacity of the method.

Implementation of this principle in gas chromatography is experimentally more challenging than in TLC, considering that the separation takes place in a tube rather than along a plane. As a result, any multidimensional separation in GC must be carried "in time" rather than "in space". In conventional heart-cut GC, only a small fraction of the 1D-GC effluent is injected to the second dimension column. In general, another fraction should not be injected into the second column before the separation of the previous fraction is finished, to prevent the possible mixing of the components of the two fractions. The number of fractions (cuts) directed consecutively to the second column can be increased by shortening the time required to finish the separation of a given fraction in the second dimension. If this time is short enough, the entire effluent from the first column can be injected sequentially into the second dimension column, which results in a comprehensive two-dimensional separation.[4]

In a true multidimensional method, the different separation dimensions applied must be orthogonal, or independent of each other. In the case of GC × GC, this implies utilizing two columns coated with stationary phases that separate compounds based on different properties. Recently, Ryan et al.

investigated the different aspects of orthogonality of a system, and discussed the use of orthogonality information as a tool in predicting two-dimensional chromatograms.[7]

A block diagram illustrating the components of a comprehensive two-dimensional GC system is illustrated in Figure 1. At the heart of the system is a modulator (interface), which periodically collects fractions or samples the primary column effluent, and injects the material into the secondary column for further chromatographic analysis. Modulators based on fraction collection are more common, therefore, the following description will apply to such modulators only.

According to Giddings' definition of comprehensive multidimensional separation, the rate of fraction collection and reinjection must be high enough to preserve the separation accomplished in the first dimension. Consequently, the interface operates as follows. Following the arrival of primary column effluent at the modulator, it collects a band of the analytes for a specified period of time. The fraction collected in this way is then reinjected into the secondary column as a narrow band. While the chromatographic separation of the reinjected analytes is occurring in the secondary column, the modulator collects a subsequent fraction of the primary column effluent. At the completion of the secondary separation, the modulator injects the next collected fraction into the secondary column. The interface's periodic action of fraction collection and reinjection is repeated throughout the entire GC × GC analysis. The detector at the outlet of the second column records a continuous linear signal, which in fact consists of a series of short second dimension chromatograms eluting from the second column in rapid succession.

Figure 2 illustrates why a modulator placed between the columns is necessary in comprehensive two-dimensional chromatographic methods. A band of analytes injected into the column (grey in Figure 2A) becomes chromatographically separated into two components (black and white in Figure 2B). The second column, coated with a different stationary phase, separates the white band into two components, but retains them more strongly than the black band (Figure 2C-D). As a result, one of the two

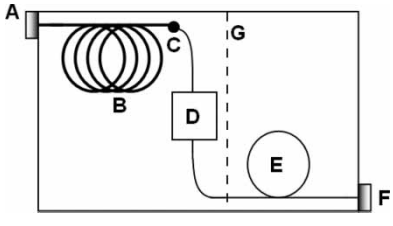

Figure 1. Block diagram of a typical GC × GC system. (A) injector; (B) primary column; (C) connector; (D) interface (modulator); (E) secondary column; (F) detector; (G) optional secondary column oven.

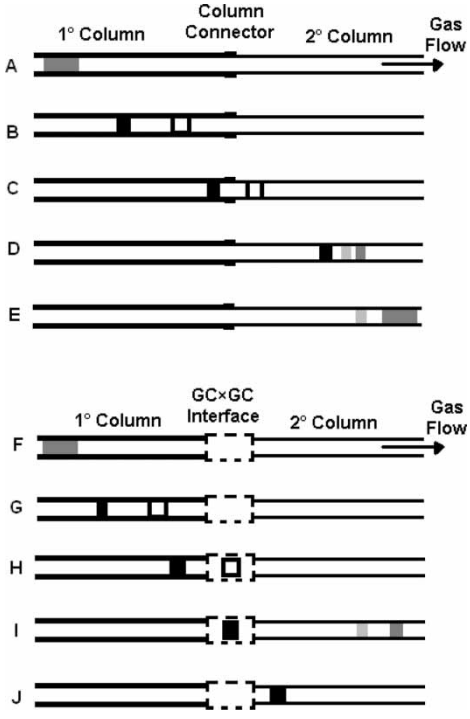

Figure 2. The role of the interface in GC × GC. A-E: columns connected in series without the interface; F-J: columns connected through the interface. See text for explanation.

previously separated analytes merges with the black band and co-elutes with it from the second column (Figure 2E). The second component of the white band elutes after the merged band. Therefore, connecting two columns in series does not qualify as a multidimensional method because the separation accomplished in the first column is not preserved.

A modulator implemented between the two columns prevents primary column effluent from continuously entering the secondary column. Periodic collection of the first column effluent and injection of the material collected into the second column helps preserve the separation accomplished in the first column. After the injection (Figure 2F), the grey band injected into the first column is separated into the white and black bands, as before (Figure 2G). The interface traps the white band for a specified period of time and reinjects it into the second column before the black band arrives at it (Figure 2H). While the white band travels along the second column (where its component(s) undergo additional separation), the black band is collected in the modulator (Figure 2I). It is injected into the secondary column only after the separated components of the white band had left the

second dimension (Figure 2J). In this way, the separation accomplished in the first dimension is preserved, and additional separation of the analytes in the second column becomes possible.

Preservation of the primary dimension chromatogram is predominantly dependent on the rate of modulator sampling or collecting. Successful preservation of the separation accomplished in the first dimension requires that each primary dimension peak be sampled at least three times,[8] although 2.5 times has also been suggested as the optimal value.[9] If the former criterion is adopted, a 12 second wide peak exiting the primary column should be sampled every 4 seconds, therefore, the modulation period should be at least 4 seconds. The effect of the modulation period on the preservation of the primary dimension has been discussed in our previous review paper.[4]

The fact that each peak eluting from the first column should be sampled at least 3 times implies that the separation taking place in the second column must be very fast. To accomplish this, the widths of the bands injected into the second column in each modulation cycle must be very small. This increases peak capacity, as more peaks can fit into the short chromatographic space available in the second dimension. In addition, narrow peaks have higher signal-to-noise ratios compared to broader peaks of the same area, which improves analyte detectability and overall sensitivity of the method. The latter is possible because background components (e.g., column bleed) collected together with the analytes of interest in the modulator can be separated chromatographically from them, therefore, an overall improvement of the signal-to-noise ratio is observed compared to one dimensional separations.

It should be pointed out, that increased signal intensity in the second dimension is only achieved with modulators that collect (focus or compress) the primary effluent, rather than sample it (see later). Readers interested in a more thorough analysis of modulation are advised to refer to papers by Marriot et al.[10] or Lee et al.[11]

GC × GC Chromatogram Interpretation

A conventional 1D-GC chromatogram is a two-dimensional plot of detector signal intensity versus retention time. A GC × GC chromatogram, however, is a three-dimensional plot with two retention times and signal intensity as the axes. Figure 3 illustrates the generation of a GC × GC chromatogram. The detector at the outlet of the secondary column records a continuous signal, being in fact a series of very short second dimension chromatograms produced by each modulation cycle (Figure 3A). In this form, the chromatogram is practically unreadable considering the sheer number of peaks present and the fact that each component eluting from the first column shows up in at least three secondary chromatograms. Thus, the linear chromatogram has to be

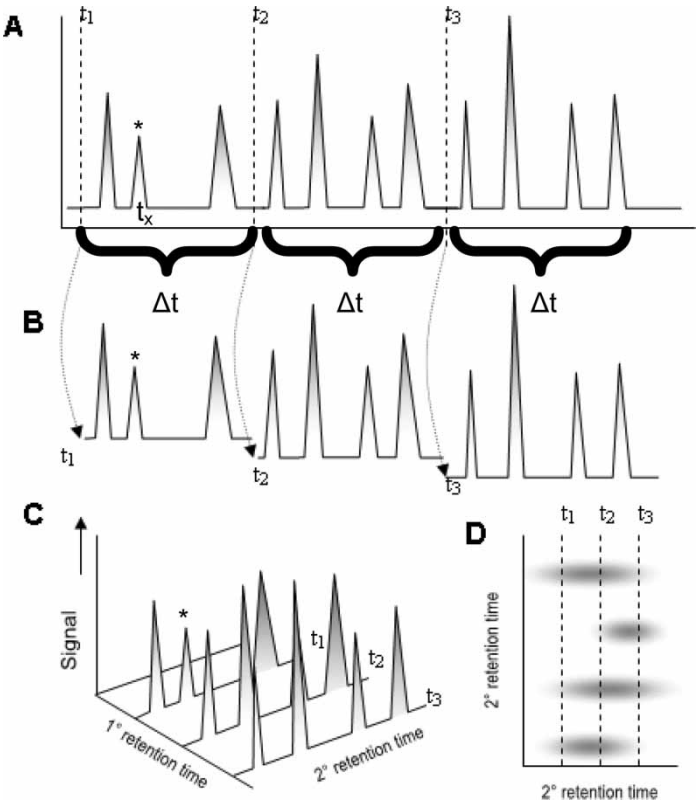

Figure 3. GC × GC data interpretation and contour plot generation. (A) GC × GC chromatogram as it is recorded by the detector; t_1, t_2 and t_3 indicate individual injection times into the secondary column; Δt is the length of the modulation period; (B) computer software separates the continuous chromatogram into individual second dimension chromatograms; (C) the software aligns the individual chromatograms side by side to create a 3-D chromatogram with primary retention time on the X-axis, secondary retention time on the Y-axis and signal intensity on the Z-axis; (D) the 3-D chromatogram viewed from the top down, which is the usual representation.

transformed into a three-dimensional representation before it can be analyzed. This task is handled by appropriate computer software, which utilizes the length of the modulation period (Δt) to cut the continuous chromatogram into the individual secondary chromatograms (see Figure 3B). The times when the injection into the second column took place (marked as t_1, t_2 and t_3) become the primary retention times for all secondary peaks eluting in a given modulation period. The secondary retention time of each peak is calculated as the difference between the absolute retention time of a given peak and the injection time for a given modulation cycle.[12] For example, for the peak marked with a star in Figure 3A, the secondary retention time is equal to $t_x - t_1$.

The individual second dimension chromatograms obtained in this way are arranged side by side at regular intervals corresponding to Δt, generating a three-dimensional plot. As illustrated in Figure 3C, the X-axis becomes the primary retention time, the Y-axis the secondary retention time, while the Z-axis represents signal intensity. In such representation, secondary peaks belonging to the same component show up in several consecutive "slices" with identical secondary retention times (recall that each peak eluting from the first dimension is sampled at least three times, therefore component(s) of this peak appear in at least three consecutive second dimension chromatograms). This feature makes it possible to recombine the individual peaks observed in the secondary chromatograms into single component peaks. The task is usually handled by the software. Each peak reconstructed in this way is characterized by two retention times and signal intensity.

GC × GC chromatograms are the easiest to visualize in three dimensions. Software used to process the data usually allows the chromatograms to be viewed from all sides and angles. This, however, is practical only when working with the chromatograms on a computer screen. Printed chromatograms are usually shown in a top-down view, resembling topographical maps (Figure 3D). In this representation, the two retention times are plotted on the X- and Y-axes, and peak intensity is colour coded. Examples of both representations of GC × GC chromatograms are illustrated in Figure 4.

The approach described requires that the length of the modulation period is precisely known and reproducible. The task can be easily handled by modern computer controlled hardware. Early on in GC × GC history, in-house written software was usually used to process the chromatograms. With GC × GC gaining popularity, however, computer software capable of calculating, plotting, and viewing this type of chromatographic information has become commercially available from several vendors. One such product, called GC Image, was designed at the University of Nebraska-Lincoln, and is available from the Zoex Corporation.[13]

In GC × GC, a potential problem arises when the secondary retention time of a compound is greater than the length of the modulation period. A peak like this (called "wraparound" peak in the literature) elutes during subsequent modulation period(s). When plotted on the 2D retention plane, it shows up with a secondary retention time shorter by the length of the modulation period (or it's multiple). Wraparound peaks are not a problem unless they co-elute with analytes of interest or interfere with the ordered nature of a GC × GC chromatogram (see later). Fortunately, in many cases they can be easily identified, as they are significantly broader than the surrounding peaks because of the much longer time spent in the secondary column. Micyus et al. developed a method for reducing the ambiguity in the determination of secondary retention times.[14]

Quantitative aspects of GC × GC are in fact very similar to those encountered in 1D-GC, as the 2D chromatograms are in fact generated from a linear chromatogram. Thus, it is easy to determine the areas of all peaks eluting from

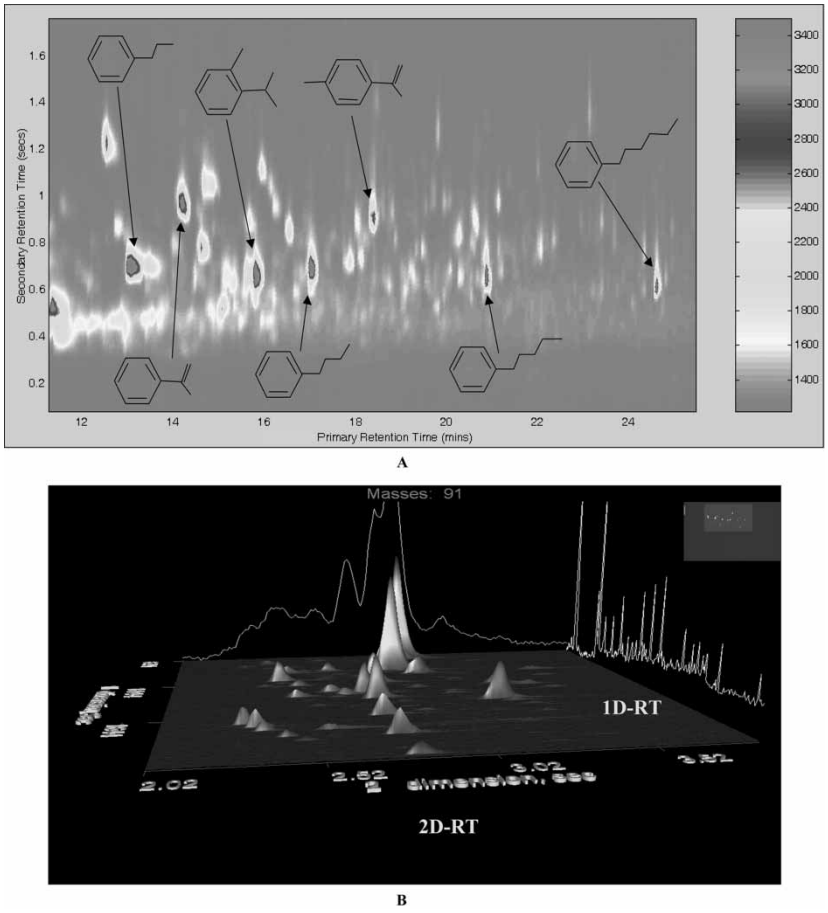

Figure 4. Examples of GC × GC chromatograms of urban air particulate matter ($PM_{2.5}$) collected on quartz filters. Small sections of the filters were thermally desorbed at 450°C and analyzed by GC × GC with Leco Pegasus IV TOF-MS detection. Contour plot of extracted ion m/z 91, characteristic of alkyl-substituted benzenes (plotted using custom made software). Each spot represents an individual compound; the color of each spot codes the abundance of that compound in the sample. Some of the small peaks are poorly visible due to the limited dynamic range of the color scale used to show peak intensity. 3-D GC × GC chromatogram of extracted ion m/z 91 plotted using Chroma-TOF software (Leco Corp.). Summation of all peaks eluting from the second dimension column at a given primary retention time leads to a reconstructed 1D chromatogram (visible on the right). These peaks would coelute in conventional 1D GC.

the second dimension column using standard chromatographic software. As long as it is clear which second dimension peaks belong to which component, the total peak area for this component can be easily calculated. This task can be very tedious if performed manually; fortunately, commercial software

packages usually have this functionality built in. Other approaches to analyte quantitation were proposed (e.g., determination of the virtual volume of a three-dimensional peak), yet the simple summation of peak areas seems to work the best.[15]

Analyte identification in GC × GC is more reliable than in 1D-GC, as each peak is described by two retention times rather than one. Readers seeking a basic understanding of peak identification in GC × GC are advised to read earlier review papers [e.g., refs. 4, 6]. Chemometric tools are often employed to help with peak identification. For example, in analyses utilizing a flame ionization detector (FID), an approach using a peak matching algorithm can be useful. Point-pattern matching is a method that uses peak location on the retention plane to match unknown peaks with known ones. This method is simple and effective because when each peak in a plane is represented as a point, a peak-pattern matching problem (three-dimensional) becomes a point-pattern matching problem (two-dimensional).[13] Numerous pattern-matching algorithms are presently being developed; one example is Recognition by Adaptive Subdivision of Transformation Space (RAST), which can be implemented into other software relatively easily.[16] Chemometric techniques can also be helpful in identifying peaks when the chromatographic conditions (e.g., oven temperature programming rate or carrier gas pressure) change from run to run.[17]

One of the most important advantages offered by GC × GC is the ordered nature of the chromatograms obtained by this method. Related compounds such as, for example, alkanes, alkenes, and aromatics in the analysis of petroleum fractions, tend to appear as distinct bands on the 2D GC plane and, therefore, can be easily recognized by experienced GC × GC chromatographers. Pattern recognition based on this feature is extremely useful in the petroleum industry. Comparative analysis via highly reproducible and ordered chromatograms has also led to successful application of GC × GC in the forensic sector. Gaines et al. illustrated the ability to compare sections of an unknown GC × GC chromatogram with chromatograms contained in a GC × GC chromatogram library, in an effort to see if two independent samples originate from the same source.[18] As of today, nearly all forensic GC analyses are based on pattern matching of 1D chromatograms; however, the advantages offered by GC × GC, such as increased resolution, enhanced sensitivity, and highly ordered nature of the chromatograms, are certain to make this method invaluable to forensic science.

GC × GC Instrumentation

GC × GC utilizes many instrumental components found in any 1D-GC instrument. For example, injectors used in GC × GC play the same role as in 1D-GC. Consequently, injector types and injection techniques in

GC × GC are selected according to the needs of the analysis in the same manner as they are in 1D-GC analyses.

The choice of columns is critical for successful GC × GC separation. The primary column is usually long, with typical dimensions of 15–30 m × 0.25 mm. The stationary phase film thickness in the primary column usually ranges from 0.25 to 1 μm (or more), to allow for the generation of peaks with widths of 10–20 s in this column. Peaks of such widths are required in order to sample each peak at least three times with the typical modulation periods of ~3 to ~6 s. The primary column is usually coated with a non-polar stationary phase; some common choices include 100% polydimethylsiloxane or 95/5 methyl/phenyl siloxane.

The second dimension column is always very short, because each individual separation in this dimension should be finished in a time shorter than the modulation period. Typical secondary column dimensions are 0.5–1.5 m × 0.1–0.25 mm. Intuitively, narrow bore columns coated with thin films of the stationary phase should allow for the most efficient and fastest separations. It is not surprising, therefore, that most GC × GC separations are carried out with 100 μm I.D. columns in the second dimension. However, recently published research results indicate that the intuitive choice might not be optimal after all. Harynuk et al.[19] demonstrated recently that peak overloading, which can be particularly severe in the second dimension because of peak compression in the modulator, might completely negate the gains associated with the use of narrow bore columns. In fact, comparable (or better) separations can often be obtained with wider bore columns (e.g., 250 μm I.D.), which are less prone to overloading. Beens et al. studied the effect of carrier gas pressure on the efficiency of separation in the first dimension.[20] He also concluded that 100 μm I.D. second-dimension columns are not necessarily the best choice for GC × GC.

Keeping in mind that any comprehensive two-dimensional chromatographic separation must rely on two different separation mechanisms, it is clear that the stationary phase of the second column must be distinctly different from that of the first. Since the primary columns are usually non-polar, the secondary columns are most often coated with polar stationary phases. Common secondary column stationary film thicknesses range from 0.1 to 0.25 μm, allowing for higher separation efficiencies via reduced retention. Typically, the stationary phases are 50/50 phenyl/methyl or "wax" (polyethylene glycol) based; though sometimes, good results can be achieved with liquid crystal, fluorinated or chiral phases. Interestingly, it is not by mere convention that the primary column is usually non-polar and the secondary column is polar. Non-polar columns separate compounds based on dispersive forces, rather than intermolecular forces.[10] Because dispersive separations increase in efficiency over time, a long non-polar column is preferred. Any compounds co-eluting after the first separation likely exhibit a range of polarities, making polarity an ideal choice for an orthogonal second

dimension separation.[10] If the setup was reversed, that is, a long polar column would be followed by a short non-polar column, the separation would be less effective because the secondary column would not be long enough to allow effective separation based on dispersive forces. This would make it inefficient in resolving compounds of similar polarities.[10]

Figure 1 illustrates that GC × GC systems can utilize an optional second oven for the secondary column; in fact, most commercially available systems house secondary columns in a separate, small oven. This option provides more flexibility in method development and allows better control of the secondary column. On the other hand, in most custom-made GC × GC systems, both columns are housed in one oven to avoid making the system too complex. Satisfactory separations can usually be achieved with both setups.

Although the same detectors used in 1D-GC can be applied in principle to GC × GC systems, the range of detectors suitable for successful analyses is somewhat narrower because any detector used in GC × GC must be characterized by a high data acquisition rate. Peaks eluting from the secondary column are in the form of short chromatographic pulses, generally shorter than 150 ms.[10] Reliable and reproducible determination of a chromatographic peak area requires that at least 10 data points are collected along the peak profile. Consequently, with 150 ms peaks, the detector should have a data acquisition rate of at least 70 Hz. Flame Ionization Detectors (FID) can acquire data at rates as high as 200 Hz, therefore, they remain the most popular choice in GC × GC separations. Apart from the FID, the following conventional detectors have been recently successfully applied in GC × GC: Electron Capture Detector (μ-ECD),[21,22] Atomic Emission Detector (AED),[23] Sulphur Chemiluminescence Detector (SCD),[24] and Nitrogen Chemiluminescence Detector (NCD).[25]However, no other detector has revolutionized chromatography in the same way as the mass spectrometer (MS). Mass spectra generated for eluting peaks in fact add another dimension to the separation. In the past, most mass spectrometric detectors were not fast enough to handle the narrow chromatographic pulses produced by the second dimension columns. For example, quadrupole mass spectrometers in the full scan mode typically could only acquire two to three spectra per second, which was woefully inadequate considering the typical 2nd dimension peak width of 150 ms. Modern, fast scanning quadrupole MS systems have scan rates as high as 10,000 amu/s, which brings them closer to the requirements of GC × GC. Such coupling was demonstrated recently by Beens et al.[26] To achieve the required data acquisition rate, a limited scan range of 50-100 mass units had to be used. Song et al. accomplished a scanning frequency of 19.36 Hz with a quadrupole MS by reducing the mass range to 42-235 amu. This was suitable for some GC × GC analyses.[27] However, by far the best choice for coupling with GC × GC is the time-of-flight MS (TOF-MS).[28–30] Two characteristics that make it more practical than any other MS in combination with GC × GC are its extremely high data acquisition rate (as many as 1,000 full

scan spectra can be acquired in one second) and its high spectral deconvolution power. A TOF-MS combined with a GC × GC system provides one of the most powerful separation tools available to analytical chemists. Even though the high costs of TOF-MS systems make them scarcely available throughout most laboratories, they found applications in many different areas of analytical chemistry (covered later).

It was suggested that because of the great resolving power of GC × GC, TOF-MS could only be used in the first run, as a tool to identify compounds in the two-dimensional retention plane. Following this, chromatograms obtained with an FID could be compared to the initial TOF-MS analysis. It is clear that the two chromatograms produced in this way would not match exactly (MS operates under vacuum outlet conditions, whereas FID operates under atmospheric pressure). Shellie et al. were successful in designing a solution to compensate for this: a T-Union was placed between the second column and the detector to provide auxiliary gas flow. Experiments in this configuration proved that the two chromatograms, one generated with the FID and the other with the TOF-MS, were essentially identical.[31] However, if one has routine access to a TOF-MS, it would be in the analyst's best interest to use this detector for all analyses because of its much better selectivity and ability to identify unknown compounds.

The modulator is the heart of any GC × GC system, and as such has traditionally been the subject of most of the research and development efforts. Presently, two classes of modulators are being used: valve-based modulators and thermal modulators. Thermal modulators can be further classified into heater-based (modulating via an increase in temperature), and cryogenic (modulating via a decrease in temperature). The next section briefly summarizes the history of interface design. Readers seeking a more thorough analysis of interface technology are advised to refer to Harynuk et al.,[4] Marriott et al.,[21] and/or Bertsch et al.[15]

Thermal GC × GC Modulators

Heater-based GC × GC modulators

The first GC × GC modulator (Figure 5A) was developed by Liu and Phillips in 1991. Positioned between the primary and the secondary columns, the interface consisted of a segment of thick film capillary column, painted with gold paint.[5] Analytes exiting the primary column were trapped in the thick film of the stationary phase, and reinjected into the secondary column periodically by resistive heating of the gold painted capillary. This modulator provided evidence that the concept of GC × GC was achievable. It also indicated that dual-stage modulation is a necessity whenever thermal modulators are used. When the entire trapping capillary was heated at once, some of the analytes exiting the primary column passed through the interface without being focused. This caused band broadening and peak

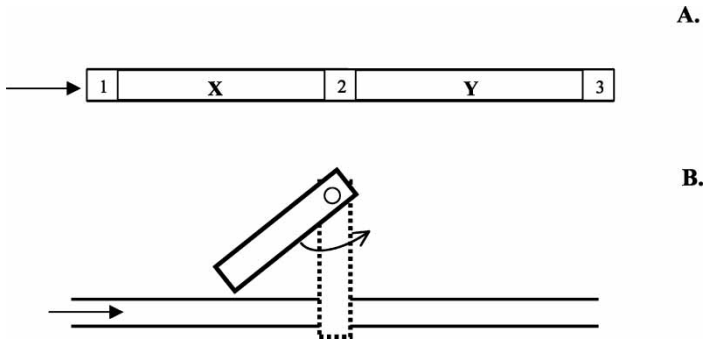

Figure 5. Schematic diagrams of (A) the original dual-stage thermal modulator,[5] and (B) rotating thermal modulator[34,35] (see text for details).

shape irregularities due to analyte breakthrough. The phenomenon was eliminated by alternately heating two segments of the capillary. Analyte(s) trapped in the first segment (X in Figure 5A) were thermally desorbed when this segment was heated by passing electric current between points 1 and 2, and retrapped in the second segment of the capillary (Y in Figure 5A). Once the first segment cooled down sufficiently to trap the analytes again, the second segment was heated to inject the analytes into the secondary column by passing electric current between points 2 and 3. In this way, analyte breakthrough in the modulator was prevented. The idea of dual-stage modulation in GC × GC is used in almost all thermal modulators these days.

The first commercially available modulator, the rotating thermal modulator (Figure 5B), was also developed by Phillips et al.[34,35] This modulator also utilized a thick film capillary for primary column effluent trapping and focusing. Injection of the trapped band into the secondary column was accomplished by the use of a rotating heater, which heated the trapping capillary to a temperature higher by about 100°C than the oven temperature. This modulator proved suitable for a wide range of applications; however, the moving parts occasionally caused problems. In addition, it was incapable of collecting volatile compounds at typical oven temperatures, and the maximum oven temperature had to be kept about 100°C lower than the maximum operating temperature of the stationary phase in the trapping capillary. Our group developed a dual-stage, solid sorbent based modulator similar in design to Phillips' first modulator.[36] This modulator had a much higher capacity for analyte trapping, but the maximum temperature limitations were similar.

Presently, heater-based modulators are rarely used. Their inability to effectively trap volatile compounds at conventional oven temperatures and the requirement for high modulation temperatures for analytes with high boiling points were the main reasons why they were largely replaced by cryogenic modulators.

Cryogenic GC × GC modulators

While research efforts in the USA resulted in heater-based modulators, Phillip Marriott of Australia became the pioneer of cryogenic modulators. His longitudinally modulated cryogenic system (LMCS), illustrated schematically in Figure 6, worked on a principle somewhat similar to that of Phillips' rotating thermal modulator.[37] However, instead of trapping the effluent at oven temperature and desorbing it by increasing the temperature, the LMCS trapped the analytes at temperatures significantly below that of the GC oven, and reinjected them at oven temperature. The interface consisted of a movable, liquid CO_2 cooled trap installed around the end segment of the primary column. As the effluent exited the primary column, its components were trapped and focused in the stationary phase of the segment of the primary column contained inside the modulator (position "R" in Figure 6). The trap was then quickly moved downstream of the trapping position (to position "T" in Figure 6), which brought the trapping segment "R" back to oven temperature and resulted in remobilization of the analytes trapped in the stationary phase. The remobilized band was retrapped in the downstream segment ("T"). Once the modulator was moved back to its initial position ("R"), the band trapped in position "T" was injected into the second column, while another portion of the primary column effluent was trapped in the upstream position. This process was repeated throughout the whole run. The dual-stage modulation in LMCS prevented both analyte breakthrough and excessive band broadening in the second dimension.

The cryogenic modulation introduced with LMCS provided key advantages over heater-based modulation. First, there was no need for additional heating, as the modulator did not need to be raised to a temperature above that of the GC oven. Consequently, the maximum oven temperature was limited only by the thermal stability of the stationary phases in the columns. Second, due to the use of cryogenic liquids for modulation, the capability to trap volatile analytes was much better, although the most volatile analytes were still not trapped efficiently at the liquid CO_2 temperature (approximately $-50^{\circ}C$). Therefore, the LMCS demonstrated that cryogenic interfaces offered a better solution to modulation than heater-based modulators.

The success of LMCS spurred more intense research efforts, in an attempt to further optimize cryogenic modulation. The next goal became the development of cryogenic modulators with no moving parts inside the

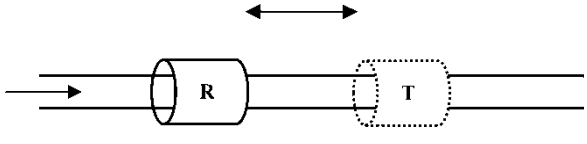

Figure 6. Schematic diagram of the LMCS system[37] (see text for details).

GC oven. One of the first cryogenic modulators with no moving parts was developed in our laboratory.[36,38] The interface consisted of two silicosteel trapping capillaries connected in series and housed in a cryochamber cooled with liquid nitrogen. The traps were alternately heated by passing pulses of electric current through them. Thus, trapping was accomplished by cooling with liquid nitrogen, and reinjection via heating by capacitative discharge.

Ledford and Billesbach presented another type of cryogenic modulator with no moving parts[39] This interface, shown in Figure 7A, ensured dual-stage modulation by alternately trapping and focusing analytes at two spots via two cold CO_2 jets (C_1 and C_2 in Fig. 7A). The trapped bands were remobilized by heating with hot air jets (H_1 and H_2 in Fig. 7A). A version of this modulator utilizing liquid nitrogen to cool the cryogen gas is now available commercially on GC × GC instruments from Leco Corporation. Beens et al. simplified the design by eliminating the hot air jets and relying on the GC oven to remobilize the bands instead (Figure 7B).[40] This modulator is used on GC × GC instruments offered by Thermo Electron Corporation. More recently, Ledford et al. simplified the concept of his original modulator by creating a single cryojet interface capable of dual-stage modulation via a delay loop (Figure 7C).[41] In the design, gaseous nitrogen cooled with liquid nitrogen is used to modulate the effluent from the first column. Presently, this is one of the simplest dual-stage cryogenic modulator designs. It is available commercially from Zoex Corporation. The main limitation of this design is that the length of the loop and the velocity of the carrier gas have to be carefully adjusted whenever the chromatographic conditions change.

A modulator based on the concept of the dual-stage, single jet interface with a delay loop was also developed in our laboratory.[42] The main difference between this design and the one developed by Ledford at al.[41] is that liquid nitrogen is used for trapping rather than cold nitrogen gas. This allows for efficient modulation of even the most volatile analytes. In addition, the cryogen delivery system was optimized to reduce the consumption of LN_2 to about 20 L/day, while commercially available systems usually consume 50–100 L/d.[42]

Recently, Sacks et al. developed yet another cryogenic modulator with no moving parts.[43] The modulator utilizes cold air for analyte trapping and resistive heating of the traps for band remobilization. Presently, it is a single-stage design, which results in analyte breakthrough during cooling of the trap. Additionally, as the trapping temperature is only $-30^{\circ}C$, it is not capable of trapping very volatile analytes. On the other hand, it is characterized by low investment and operating costs.

Valve-Based GC × GC Modulators

The first valve-based modulator based on a fast switching diaphragm valve was developed by Synovec and coworkers.[44] In this design, the effluent

from the primary column was diverted to the atmosphere for most of the analysis time. The diaphragm valve was periodically actuated for a very short time to direct a small amount of the effluent to the second dimension column, where separation took place with the help of auxiliary carrier gas

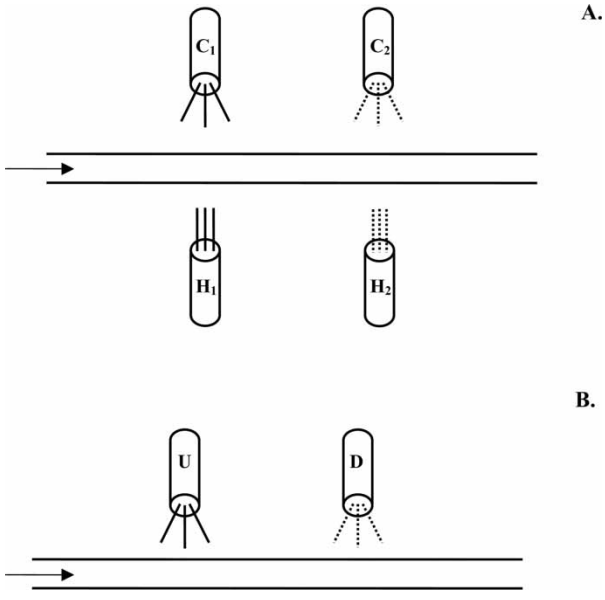

Figure 7. Schematic diagrams of selected dual-stage cryogenic modulators. (A) Cryogenic modulator developed by Ledford and Billesbach.[39] When the upstream cryojet (C_2) is on and the downstream cryojet (C_1) is off, analytes exiting the primary column are trapped and focused in the downstream position. The upstream cryojet is then turned on to prevent breakthrough from the primary column. The band trapped downstream is injected into the second column by turning the downstream cryojet off and the downstream hot air jet (H_2) on. Material trapped upstream is transferred to the downstream trapping spot by turning cryojet C_1 off and hot jet H_1 on, with cryojet C_2 on. The cycle is repeated throughout the run. (B) Cryogenic modulator developed by Beens et al.[40] At the beginning of the modulation cycle, liquid CO_2 from the downstream jet (D) traps and focuses analytes exiting the primary column. When this jet is turned off, analytes are injected into the secondary column. Breakthrough is prevented by simultaneously turning the upstream jet (U) on. The cycle is repeated throughout the run. (C) Single cryojet, dual-stage modulator with a delay loop.[41] When the cryojet (C) is turned on, the analytes eluting from the primary column are trapped in position 1. The cryojet is then turned off and the hot jet (H) is turned on, which causes the analytes trapped in position 1 to be launched into the delay loop. The cryojet is turned back on (and the hot jet is turned off) before the band exits the loop, so that the analytes exiting the delay loop are refocused in position 2. In the subsequent cycle, the band trapped in position 2 is launched into the secondary column, while the eluate from the primary column is trapped in position 1.

(*continued*)

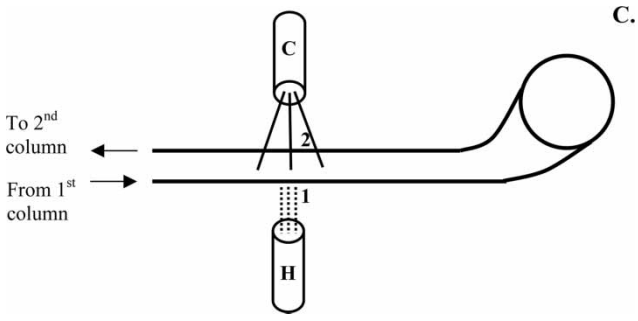

Figure 7. Continued.

supplied through the valve. This method was significantly less sensitive than the methods based on thermal modulation, as only 10 to 20% of the primary effluent was subjected to the second dimension analysis. In addition, the range of applications was limited by the relatively low maximum operating temperature of the diaphragm valve.

Seeley et al.[45] developed an improved version of this interface by incorporating a sample loop in the valve. In this technique, the effluent from the primary column was directed to the atmosphere through the sample loop. Once the loop was filled, the valve was switched, and the fraction collected was injected into the second dimension column, operated at flow rate at least 20 times higher than the flow in the primary column. Owing to this, the gas in the sample loop was physically compressed and injected as a narrow pulse to the second dimension column. This method was characterized by better sensitivity, as approximately 80% of the effluent from the primary column was sampled.[45]

The same group came up with a design which eliminated the need for a high temperature valve.[46] The interface used two sample loops and a pneumatic switching system, which allowed the effluent from the primary column to be collected in one loop, while the fraction collected in the other loop was transferred to the second dimension column and underwent separation there. Once the first loop was filled completely with the effluent, pressure balance in the system was changed by switching a valve mounted outside of the GC oven. This directed the effluent from the first loop to the secondary column, while the effluent from the first column was directed to the second loop. This was the first valve-based modulator which subjected 100% of the sample to separation in both dimensions, without venting any of it to the atmosphere. It also did not suffer from the temperature limitations imposed by the diaphragm valve. The main disadvantage of this approach is the fact that only very short modulation periods can be used (<2 s), therefore, the separation power achievable in the second dimension might be somewhat limited. Nevertheless, the modulator was demonstrated to perform well in the determination of aromatics in gasoline.[47]

Valve-based modulators are used less frequently than thermal modulators, even though they are simpler and often do not require any consumables. Initially, the reluctance in broader acceptance of the technology was mainly related to the fact that only a small fraction of the first column effluent was subjected to separation in both dimensions. Recent advances in the technology largely eliminated this limitation. While some problems still remain, it is conceivable that in the future, valve-based modulators might play a much more significant role than they do today.

APPLICATIONS

Although it is a relatively young method, GC × GC has established itself as a reliable tool capable of generating very high resolution separations. It offers significant advantages over conventional 1D-GC, and in some cases even GC-MS. Among these advantages are enhanced resolution,[28,48,49] increased peak capacity[6, 50] improved sensitivity.[25,29,30,51–53] and ordered structure of the chromatograms,[28,54] which is invaluable in analyses based on pattern recognition. The range of applications of the method spans from petrochemical and environmental analysis to food and fragrance, health, and forensic science. Table 1 summarizes selected examples of such applications. This paper is not intended to be a comprehensive review of the technique, therefore, readers seeking a more thorough overview of GC × GC applications are encouraged to refer to earlier literature.[26,55,56]

Initially, the main application of GC × GC was the analysis of petroleum products. Typical petrochemical samples, including gasoline, diesel, kerosene, and oils, can contain thousands of components. Increased peak capacity, enhanced resolution, and the ordered nature of GC × GC chromatograms improved both the qualitative and quantitative aspects of petrochemical analyses. The technique produced the best chromatographic separations ever achieved for these types of samples, and allowed quantitative analysis of many previously undetected compounds in complex matrices. The highly ordered nature of GC × GC chromatograms offered structural elucidation of the different components in the various petroleum samples. For example, components of petroleum fractions could be easily separated into different families such as paraffins, naphthenes, and aromatic,s ranging from C10 to C17.[57] In fact, at that time, advances in GC × GC technology could be measured by observing improvements in 2D chromatograms generated by petrochemical samples. More recently, the field has witnessed the introduction of less common detectors in petrochemical analysis by GC × GC. For example, the sulphur chemiluminescence detector (SCD) was successfully applied in analyses attempting to identify and quantify sulphur containing compounds in crude oils,[24] and nitrogen chemiluminescence detector (NCD) was used to quantify nitrogen containing compounds in diesel fuels.[25] In the latter case, the presence of indoles and carbazoles could be demonstrated in fuel

Table 1. An overview of applications and advantages offered by GC × GC to various fields of research (updated from reference[4]).

Application	Advantage over 1D GC	Reference
Petrochemical/ organics	Greatly enhanced separation power. Highly ordered, structured chromatograms simplify group-type analysis.	[18,24,25,48,57, 58,63–75]
Foods and fragrance	Greatly enhanced separation power. Allows determination of trace analytes not detectable in 1D separations. Simplified sample preparation in target analysis if the analytes can be chromatographically separated from matrix components.	[50–53,60, 76–86]
Environment and health	Greatly enhanced separation power. Potential gains in sensitivity. Simplified sample preparation may be possible. Multiple 1D analyses can be replaced with a single GC × GC analysis (e.g. in PCB analysis).	[22,28,87–104]
Forensics	Greatly enhanced separation power. Highly ordered and structured chromatograms provide more detailed patterns to be used in pattern recognition/comparison studies.	[18,29,105,106]

samples. These compounds were very difficult to detect and analyze by 1D-GC because of the generally low total nitrogen content of fuel samples. It seems that GC × GC has now been generally accepted as a powerful and reliable analysis method in the petrochemical sector; hence, the number of publications has dropped compared to the recent past. This, however, does not indicate that focus has shifted away from these types of analyses, as they are still being pursued in many laboratories. Some recent examples include papers by Diehl et al.[48] and Johnson et al.[58]

In many cases, successful environmental analysis requires the identification and quantification of a trace compound within a complex matrix. Increased peak capacity and enhanced resolution of GC × GC allow for the separation and identification of many environmentally relevant compounds, while increased sensitivity helps with quantification of those compounds. For example, polychlorinated biphenyls (PCBs) have received much attention because they are suspected human carcinogens and are subject to bioaccumulation and biomagnification. PCBs are very complex mixtures, consisting of up to 209 different congeners (based on the position and number of chlorine substitutions). No single 1D-GC method, even when linked to the most powerful MS detectors, is capable of complete separation and quantitation of all the congeners. Recently, Bordajandi et al.[22] and Focant et al.[28,59] used the great separation

power offered by GC × GC in conjunction with μ-ECD and TOF-MS detection, respectively, to improve the separation of PCB congeners. Focant et al. were able to completely resolve 192 of the 209 congeners (more than 91%); only eight co-elutions occurred between the remaining 17 congeners.[28]

The food industry has also benefited from the same advantages GC × GC offers to environmental analysis. For example, the technique was recently successfully applied to the analysis of volatiles in strawberries[52] and roasted coffee beans.[60] It is important to note that in both studies, the analytes of interest were present in trace amounts in very complex matrices. In fact, some of the analytes determined with GC × GC could not be detected at all with conventional 1D-GC methods. The technology was also successful in analyzing volatile oils in complex herbal mixtures, composed of ginseng in combination with other herbs.[51] Mayadunne et al.[53] analyzed and quantified amino acids in food and beverage products; it should be noted that amino acids, unless isolated in solution, are rarely and unsuccessfully analyzed with 1D-GC-MS methods.

Many fragrance related analyses have benefited from the advantages offered by GC × GC. For example, the European Commission's Scientific Committee on Cosmetics and other Non-Food Products identified 24 chemicals found in perfuming agents as skin sensitizing.[61] Shellie et al. used GC × GC-FID to detect and quantify these substances.[50] Complete separation of all 24 chemicals was accomplished in just 30 minutes. However, their determination in three different fragrances was somewhat less successful, as it was found that some coelutions with matrix components caused problems. The use of mass spectrometry as the third separation dimension was suggested to solve this problem.

In the area of forensic science, application of GC × GC to drug screening and specific drug confirmation was recently described by Song et al.[29] Most of the 78 drugs of interest in forensic science were separated and identified in one analysis by coupling GC × GC to TOF-MS. As such results were not previously achievable with 1D-GC, even when coupled with the most powerful MS detectors, it seems fair to predict that such accomplishments will create many more opportunities for GC × GC in this area of science.

Applications previously impossible with 1D-GC methods become reality with the implementation of GC × GC. For example, the method can be used to directly study molecular interconversion during GC analysis. Marriott et al.[62] studied the effect of different column phases on interconversion. Based on this study, he concluded that interconversion of oximes occurs largely in the stationary phase.

CONCLUSIONS

Comprehensive two-dimensional gas chromatography is still very much a method under development. Reliable commercial GC × GC systems started

appearing on the market only a few years ago. One of the biggest challenges still lying ahead is the development of user friendly software capable of handling and interpreting the enormous amounts of data produced by GC × GC separations, especially in combination with mass spectrometry. Nevertheless, the inherent advantages of the technique, including dramatically increased peak capacity, enhanced resolution, improved sensitivity, and the ordered nature of the chromatograms are so significant that it seems only a matter of time before GC × GC becomes as routine a method as 1D-GC is today. We have already witnessed spectacular examples of separations that could not even be dreamt about in a not so distant past. With the ever expanding range of GC × GC applications, we can expect many more such success stories.

ACKNOWLEDGMENTS

The authors wish to thank the Natural Sciences and Engineering Research Council (NSERC) of Canada and the Centre for Research in Earth and Space Technologies (CRESTech) for providing funding for this research.

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Received September 30, 2005
Accepted December 15, 2005
Manuscript 6770C